Identification of good quality, viable seeds: An essential step towards induction of somatic embryogenesis of a sedge species important for land rehabilitation

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Introduction

Sedges (Cyperaceae) are key species comprising a significant ground cover component of pre-mined ecosystems in the jarrah (*Eucalyptus marginata*) forests of Western Australia. Re-establishment of sedges into disturbed sites is often problematic because many are impossible to grow from cuttings or divisions, and are extremely difficult to propagate from seed due to difficulty in collecting large numbers of seed, poor seed viability and complex dormancy mechanisms (Meney et al. 1990). Propagation by conventional techniques is often labour-intensive and time-consuming, hence the development of *in vitro* propagation methods including somatic embryogenesis (Panaia 2006). Initial efforts to extract seed embryos for somatic embryogenesis in several species have been thwarted by what appears to be erratic seed quality. We report here investigations undertaken on the sedge species *Lepidosperma squamatum* to develop a method to identify good quality, viable seed and determine to what extent somatic embryogenesis is dependent on seed quality.

Materials and Methods

L.squamatum can have up to three different aged seed spikelets on any given plant, at any one time. It is therefore important to distinguish between "old" (grey), "current" (brownish/red) seed spikelets and the "new" tender seed spikelets (pale brown) before harvesting seeds. Viable seeds were initially selected using a floatation test. Seeds were put into sterile deionised water (sdw) with 2-3 drops of Tween 80, shaken and allowed to stand. The seeds that sank were further examined for the presence of an endosperm using x-rays (Faxitron X-ray Specimen Radiography System).

Germination of viable seed did not occur under *in vitro* growth conditions [25°C in darkness on half strength Murashige & Skoog (MS)] (data not shown). The zygotic embryo of seed soaked in sdw for 2 days was excised and transferred to half strength MS supplemented with 1 uM thidiazuron (TDZ) or 1 uM 2,4-dichlorophenoxyacetic acid (2,4-D), to stimulate either germination or direct production of somatic embryos (using methods by Panaia et. al. 2004a,b).

Results

A high proportion of seed with endosperm could be selected using the flotation test as they sank, whilst seeds that floated were generally empty or had an aborted or shrivelled endosperm. The Faxitron Xray system clearly showed seeds with a full endosperm.

Primary somatic embryogenesis was achieved using excised zygotic embryos for *L. squamatum* on 1/2MS supplemented with 1 uM 2,4-D (Table 1). Although the number of somatic embryos produced was low (a total of ~ 52), these were subsequently used for secondary embryogenesis with a total of 95, 212 and 124 secondary somatic embryos produced in four weeks (Table 1).

Table 1. The mean number of primary somatic embryos produced (using viable zygotic embryos) for *L. squamatum* after five weeks incubation on treatment media and the mean number of secondary somatic embryos when using the primary somatic embryos as the explant source after four weeks incubation.

Treatment	Mean No. of Primary Somatic embryos (se mean)	Mean No. of Secondary Somatic embryos (se mean)
1/2MS	0 (0.0)	3.167 (0.7335)
1/2MS + 1 uM 2,4-D	1.733 (0.3586)	7.067 (1.2935)
1/2MS + 1 uM TDZ	0 (0.0)	4.133 (1.0523)

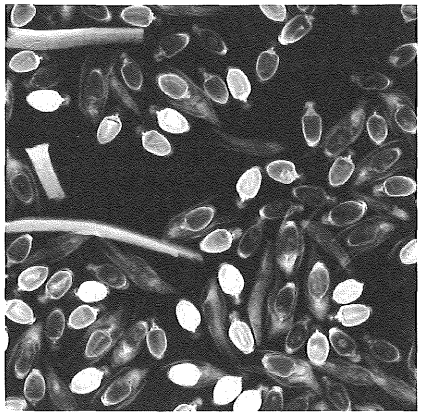

Figure 1: X-ray image of *Lepidosperma squamatum* seeds clearly showing filled seed (solid white with endosperm with possible embryo) and non-viable seed (lighter in colour with either empty or dried endosperms).

Discussion

Visually distinguishing viable from non-viable seed has proven to be extremely difficult. Investigation of plants in the field has determined that *Lepidosperma squamatum* has multiple seed spikelets of varying ages on any given plant, at any one time. These include the previous year's spikelets, the current season's spikelets and the "new" developing spikelets. Therefore, while it may appear good practice to harvest from all of the three different spikelets available to maximise seed collection, this is in fact highly inefficient, as all viable seed drop to the ground at maturity leaving behind only non-viable seed. Importantly, the harvesting of the "new" underdeveloped spikelets severely restricts seed set for the following season. The correct identification of the appropriate (current season's) seed spikelet for *L. squamatum* is essential to optimise the collection of viable seed. The subsequent testing of seed using a floatation test followed by x-ray examination, allowed selection of good quality, viable seed for experiments on somatic embryogenesis. Without this preliminary screening of seed, much time is wasted in attempting to extract embryos from non-viable seeds.

Flowering of most *Lepidosperma* species occurs two weeks following "adequate" winter rains, but seed is not mature until October/November of the same year. It may be necessary to bag the spikelets to 'catch' any viable seed that would otherwise drop to the ground. If frequent monitoring is an option, seed development can be checked and mature spikelets collected in their entirety before the viable seed begins dropping. Mature spikelets can then be kept in a dry room within trays for several days to allow the mature seed to fall out of the inflorescences. Our experience indicates that it is important that seed spikelets are not put through a thresher or cleaner as this simply dislodges the non-viable seed from the spikelets. Initial work showed seeds that looked mature and superficially similar did not have a high proportion of viable embryos. This has a significant impact on percentage germination results and efforts to extract embryos for *in vitro* culture. Successful somatic embryogenesis was only possible after the correct identification of viable seed from field collected material and may play a vital role in the mass propagation of this species for land rehabilitation.

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